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THE ROLE OF PROSTAGLANDINS IN RABBIT MONOARTICULAR ARTHRITIS

A. BLACKHAM, J.B. FARMER, H. RADZIWONIK & J. WESTWICK[1]
Department of Pharmacology and Biochemistry, R. & D. Laboratories, Fisons Ltd, Loughborough, Leicester

1 Old English (OE) rabbits produced more severe monoarticular arthritis (MAA) after sensitization and intra-articular challenge with ovalbumin than did either New Zealand White (NZW) or Dutch rabbits. NZW rabbits were better responders than Dutch rabbits.

2 The swelling of the joint in all three strains of rabbits was triphasic. There was an initial acute swelling which appeared to peak at 2-4 days after challenge. This was followed by a decrease in joint size, and then a secondary increase in size beginning 1-2 weeks after challenge.

3 An investigation of MAA in OE rabbits showed that there was an increase in E-type prostaglandins, total leucocyte counts and free acid phosphatase activity in the synovial fluid of the challenged joints at 6 h, 19 h, 47 h, 7 days and 46 days following challenge. There were also histopathological changes at these times. In addition, there was an increase in the surface temperature of both the challenged and non-challenged knees, and a rise in the body temperature.

4 Prostaglandin levels peaked at 19 h and were equivalent to 19 ng E_2 per joint. In a separate experiment, the prostaglandin present at 18 h was shown to be mainly E_1. Maximum levels of prostaglandin appeared to coincide with maximum joint temperature, but preceded maximum joint swelling and a significant rise in both the number of inflammatory cells and the free acid phosphatase activity in the synovial fluid, all of which occurred at 47 hours.

5 Indomethacin, 7.5 mg/kg orally twice daily, almost completely inhibited the increase in prostaglandin levels in the challenged joints and produced a moderate reduction in joint swelling. It also reduced the increased surface temperature of both knee joints and the raised body temperature. However, indomethacin had no effect on the number of leucocytes present, the free acid phosphatase levels, or the histopathological changes in the joint.

6 The mean plasma level of indomethacin ranged from 0.5 to 3 μg/ml at the time when the animals were killed.

7 Lysosomal enzymes may be more important than prostaglandins in rabbit MAA, and the lack of effect of indomethacin on joint histopathology may be due to its inability to prevent the release of these enzymes.

Introduction

Dumonde & Glynn (1962) first described monoarticular arthritis (MAA) in rabbits sensitized with a suspension of heterologous fibrin in Freund's complete adjuvant and subsequently challenged intra-articularly with fibrin. However, it was later shown that fibrin could be replaced by another antigen, ovalbumin, which is obtainable in a pure and soluble form (Consden, Doble, Glynn & Nind, 1971). The result of this procedure is the production of a progressive immunological arthritis, which in histopathological terms closely resembles rheumatoid arthritis in man.

It has been recently proposed that aspirin-like drugs exert their clinical anti-inflammatory activity by inhibiting prostaglandin synthesis (Vane, 1971). However, the presence of prostaglandins has not been reported in conditions such as rheumatoid arthritis, in which these drugs are used. Since rabbit MAA is a chronic arthritis which closely resembles rheumatoid arthritis, we have investigated the presence of prostaglandins in this animal model, and tried to determine their importance by using indomethacin, which is a potent inhibitor of prostaglandin synthetase (Vane, 1971). Following initial observations of prostaglandins in the joints of rabbits with MAA (Blackham, Farmer, Radziwonik & Westwick, 1973), we have tried to correlate their presence with other parameters of inflammation including joint size, joint temperature, leucocyte migration,

acid phosphatase levels and joint histopathology.

Methods

Production of monoarticular arthritis in rabbits

Groups of 9-10 male or female twelve-week old New Zealand White (NZW), Old English (OE), or Dutch rabbits were sensitized with an emulsion of 10 mg ovalbumin in saline (0.9% w/v NaCl solution) and Difco incomplete adjuvant to which had been added 2 mg finely ground *Mycobacterium tuberculosis*. A sample of the emulsion (1 ml) was injected intradermally into five sites at the back of the animal's neck. Three weeks later the animals were skin tested with 1, 3 and 10 μg ovalbumin in saline (0.1 ml) injected intradermally into a shaved area of skin on the back of the animals. The increase in skin thickness, measured 24 h later, gave an indication of the delayed cell-mediated response to the antigen. A dose-related increase in skin thickness, measured with a micrometer, was usually observed, and all the animals injected with 10 μg ovalbumin showed at least 100% increase in skin thickness. The knee joints were then shaved, washed with 5% Savlon in IMS and anaesthetized locally with 5% xylocaine ointment. A sterile solution of 5 mg ovalbumin in 1 ml normal saline was injected through the suprapatellar ligament of the right knee with a gauge 25 needle. The left knee was injected with 1 ml sterile saline and acted as control.

Indomethacin treatment

Five groups of 9-10 male or female OE rabbits in the weight range 1.7-2.9 kg were sensitized and challenged as described previously. Four to five of the animals in each of the first four groups were treated with indomethacin, 7.5 mg/kg orally, 1 h before challenge and then twice daily for the remainder of the experiment. The remaining animals received a similar volume (0.5 ml/kg) of drug vehicle, 0.05% Tween 80 in distilled water, for control purposes. Drug or vehicle was administered 1 h before joint measurement or 1 h before the animals were killed. Groups 1-4 were killed at 6 h, 19 h, 47 h and 7 days after challenge. The animals in group 5 were dosed as above with either indomethacin or vehicle from days 38-46 and then killed.

Measurement of joint diameter

The knee joints were measured across the latero-medial aspect of the articular junction with steel calipers calibrated to read in 0.02 mm divisions. The joints were measured periodically throughout the course of the experiment. The increase in the size of the challenged joint was calculated as the difference in size of the test and control joints expressed in mm.

Measurement of joint and body temperature

The skin temperature of the anterior aspect of the joint at the articular junction was measured with a Heimann KT 13 radiometer which has a temperature resolution of $\pm 0.25^{\circ}$C within one second. The joint temperatures were recorded on a Vitatron electronic pen recorder immediately before the joint diameters were measured. Body temperature was measured with a rectal probe coupled to an electric thermometer (Light Laboratories).

Collection of synovial fluid

Just before the animals were killed both the challenged and control knee joints were injected with 0.2 ml 0.01% heparinized normal saline. The rabbits were killed by a blow on the head followed by exsanguination from cut blood vessels in the neck. Blood was collected in heparinized pots for plasma indomethacin determinations. The knee joints were then carefully dissected by cutting through the quadriceps tendon and then along the lateral and medial aspects of the capsule as the flap of tissue was pulled forward. The synovial fluid was washed out at repeated intervals with 0.1 ml heparinized saline so that the final volume of the washout was 1.2 ml. The washouts were stored in stoppered plastic microtubes in ice until centrifuged. A 0.1 ml sample was retained for total and differential cell counts and the remainder centrifuged at 1,200 *g* for 15 minutes. A 0.2 ml cell-free sample and the cell plug were used for determination of free and bound acid phosphatase activity, respectively. The remaining supernatant was either pooled for prostaglandin extraction (per group of 4 or 5 rabbits), or 0.2 ml removed before pooling and used for individual direct biological assay. Samples were stored at -20°C until assayed.

Detection and biological assay of prostaglandins

The presence of prostaglandins was detected and measured by a cascade system similar to that described by Ferreira & Vane (1967). This consisted of three isolated tissues, rat stomach strip, chick rectum and rat colon, superfused with aerated Krebs solution at a flow rate of 6-7 ml/min, and which contained: mepyramine 0.1 μg/ml, methysergide 0.2 μg/ml and atropine 0.1 μg/ml, to block the effects of histamine,

5-hydroxytryptamine and acetylcholine, respectively. Contractions of the rat colon were measured auxotonically (Harvard heart/smooth muscle transducer), while the contractions of rat stomach strip and chick rectum were measured isometrically (Grass force displacement transducers). The impulses were amplified and recorded on a Devices M19 electronic pen recorder. Under these conditions the tissues responded differentially to E and F-type prostaglandins, and were sensitive in certain instances to doses as low as 1 ng E_2 (stomach strip preparation). Standard prostaglandins (Cambrian Chemicals) were injected before the pump in a volume of 0.5 ml and individual samples of cell-free synovial washout were injected as 0.2 ml aliquots. Prostaglandin-like responses produced by the samples were bracketed wherever possible with responses to prostaglandin standards.

Pooled group samples were acidified to pH 3 with 0.1 N HCl and were extracted twice with ethyl acetate and the organic phase separated and then blown off by a stream of N_2. The remaining solids were reconstituted in Krebs solution and the prostaglandin content estimated biologically in the assay system described previously.

Separation of prostaglandins

Ten OE rabbits were sensitized, challenged and killed 18 h later. The pooled cell-free washouts from the challenged or control knee joints were extracted as previously described. The dried extracts were taken up in 0.1 ml ethanol : ether (1 : 1) and applied to thin layer chromatography plates (t.l.c.) coated with a mixture of 30 g silica gel G (E. Merck) in 60 ml water containing 3 g silver nitrate. The extracts were co-chromatographed with standard prostaglandins E_1 and E_2 by the AII system of Gréen & Samuelsson (1964). The standard prostaglandins E_1 and E_2 were visualized by spraying the dried plates with distilled water. Prostaglandins E_1 and E_2 were eluted from the chromatoplate by scraping the zones corresponding to the simultaneously developed standards into water, adjusting to pH 3 with acetic acid and extracting the prostaglandins into ethyl acetate. The extracts were then assayed biologically as described previously. The recovery of standard prostaglandin was 50-60%, estimated spectrophotometrically at 278 nm as prostaglandin B after treatment of the E prostaglandin with 0.5 N ethanolic NaOH.

Measurement of acid phosphatase activity

Cell-free aliquots of the synovial washouts (0.2 ml) were diluted to 1.0 ml with water. The corresponding cell plug samples were lysed with water, the solids spun down at 1,200 *g* and the supernatants made up to 1.0 ml.

Duplicate enzyme samples (0.2 ml) were incubated for 3 h at 37° C with 0.4 ml disodium β-glycerophosphate (Sigma), 5 mg/ml, in 0.1 M sodium acetate, pH 5.0. Reactions were stopped by addition of 0.3 ml 15% trichloroacetic acid (TCA). Free phosphate in the enzyme samples was determined by addition of TCA before substrate. After the addition of TCA, precipitated protein was removed by centrifugation at 10,000 *g* at 0° C. The phosphate content of the supernatant was assayed by a Fiske-Subbarow molybdate method modified to a microscale. Phosphatase activity is expressed as nmol PO_4 released per 3 hours.

Joint histopathology

At the end of the experiment the flesh was trimmed from the knee joints. The dissected joints were fixed in formol saline and decalcified by incubation with disodium edetate at 50° C for up to 6 weeks. The tissues were mounted in paraffin wax, sectioned in various planes and stained with eosin and haematoxylin. The histopathological changes in the joint space, synovial intima, in and around the blood vessels and in the cartilage, were graded as nil, mild, moderate or severe by an overall subjective assessment of the joint.

Measurement of plasma indomethacin levels

Plasma levels of indomethacin were determined by the method of Holt & Hawkins (1965).

Statistics

Results were analysed by Student's *t* test and were regarded as significant if $P < 0.05$.

Results

Monoarticular arthritis in Old English, New Zealand White and Dutch rabbits

Joint swelling was measured for up to 53 days following challenge and the rabbits were killed on day 56. OE rabbits showed greater joint swelling than the NZW rabbits, which in turn gave a better response than the Dutch rabbits (Figure 1). The swelling was triphasic in all three strains of rabbits. An initial acute swelling appeared to peak 2-4 days after challenge and then decreased. A prolonged secondary increase in joint size started approximately 1-2 weeks after challenge. Joint histopathology also confirmed that OE rabbits

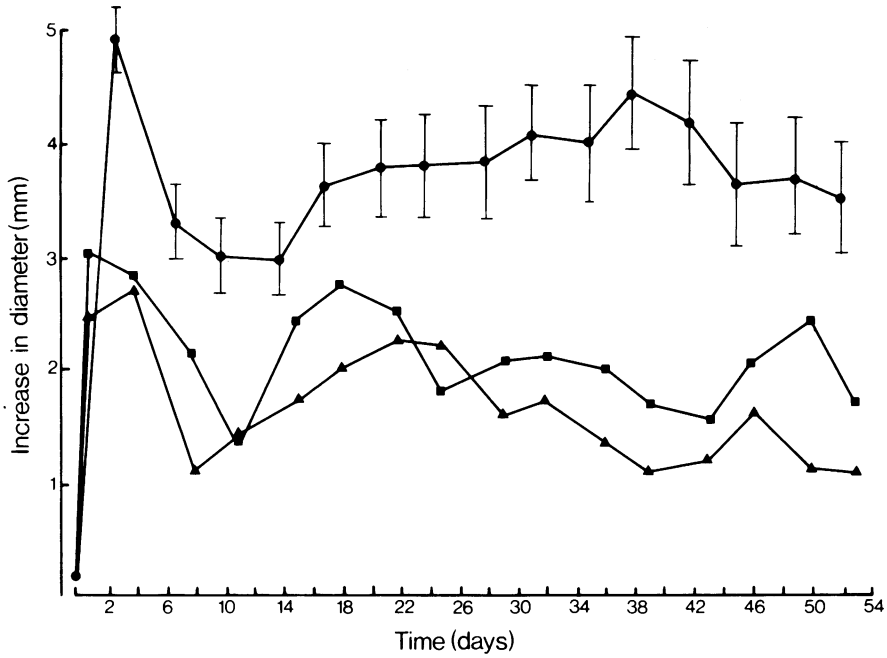

Fig. 1 Joint swelling in different strains of rabbits with monoarticular arthritis. Each point represents the mean increase in joint diameter (mm) of the challenged joints compared with the non-challenged joints in 8-10 animals. Vertical bars are s.e. of mean. (●) Old English; (■) New Zealand White; (▲) Dutch.

responded better than NZW rabbits, and NZW rabbits better than Dutch rabbits (Table 1). OE rabbits were therefore used in the following investigation.

Joint swelling

The joint swellings of the five groups of OE rabbits killed at various intervals after joint challenge are illustrated together with three indices of change in synovial fluid composition; prostaglandin concentration, total and differential leucocyte counts and free and bound acid phosphatase activity (Figure 2). The joint swellings observed in this experiment paralleled those seen in the previous experiment. Indomethacin produced a moderate reduction in joint swelling which was significant on day seven.

Synovial prostaglandins

The mean prostaglandin concentrations in the bulked and extracted synovial samples are illustrated in Fig. 2, since the 0.2 ml individual aliquots for direct bioassay contained insufficient prostaglandin for detection except at 19 hours.

Table 1 Histopathology of challenged joints in Old English (OE), New Zealand White (NZW) and Dutch rabbits killed 56 days after challenge

Strain	No. of joints	Histopathology score			
		Nil	Mild	Moderate	Severe
OE	9	0	1	4	4
NZW	8	2	2	0	4
Dutch	9	4	1	4	0

Histopathology score based on a subjective assessment of the degree of damage and cell infiltration in the joint. One rabbit from each group died during the course of the experiment.

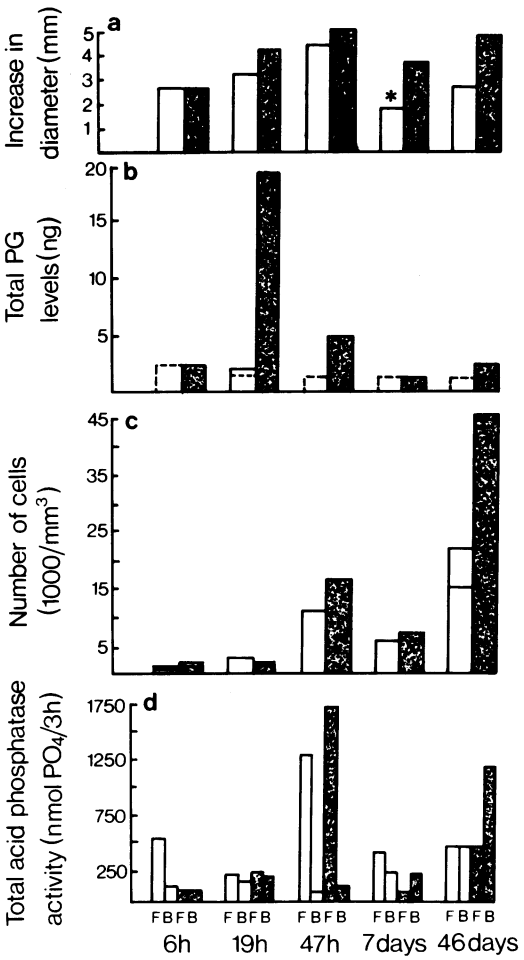

Fig. 2 Effect of indomethacin (7.5 mg/kg) or vehicle twice daily by mouth on joint swelling (a), synovial prostaglandin (PG) levels (b), total and differential cell counts (c) and free (F) and bound (B) acid phosphatase activity (d) of the challenged knee joints of Old English rabbits. Each histogram represents the mean observations from 4-5 rabbits killed 6 h, 19 h, 47 h, 7 days and 46 days after challenge. The open columns represent challenged joints in indomethacin-treated, and the stippled columns challenged joints in vehicle-treated rabbits. In (a) the joint swelling is the difference in diameter (mm) between the challenged and the non-challenged joints. In (b) prostaglandin levels are of bulked and extracted synovial fluid and the broken lines refer to the minimum levels of prostaglandin detectable in the bioassay procedure. In (c) the upper portion of each column represents the number of mononuclear cells present, and the lower portion of each column the number of polymorphonuclear leucocytes. * $P < 0.05$ for indomethacin versus vehicle.

Prostaglandin-like activity was assayed on the rat stomach strip as E_2. No prostaglandins could be detected in the control joints in either the indomethacin- or vehicle-treated groups.

Prostaglandin activity in the challenged joints of vehicle-treated rabbits rose to a maximum of 19.2 ng/joint at 19 h, and then fell to almost undetectable levels. Treatment with indomethacin reduced prostaglandins in the corresponding groups to near or below detectable levels at all times.

Total and differential leucocyte counts

The total leucocyte count in the challenged joints of the vehicle-treated animals was roughly parallel to the course of joint swelling, with a significant increase in numbers at 47 h compared with 19 h and a further increase at 46 days (Figure 2). There was a gradual increase in the proportion of mononuclear cells to polymorphonuclear leucocytes (PMNs) as the disease progressed, but the absolute numbers of PMNs increased throughout the experiment. Indomethacin appeared to reduce the number of cells in the challenged joints, but the effect was not significant. Relatively few cells ($\simeq 300/mm^3$) were observed in the control joints of either the indomethacin- or vehicle-treated animals at any time.

Acid phosphatase activity

Free acid phosphatase activity roughly paralleled total leucocyte counts (Figure 2). In addition, as might be expected, the amounts of bound acid phosphatase also paralleled the total cell counts, except at 47 h when 4-5 times as much free acid phosphatase activity, than measured on other occasions, appeared in the fluid.

Indomethacin did not significantly reduce the levels of acid phosphatase, calculated either free in the fluid or bound in cell granules, at any of the times measured.

Identification of prostaglandins

The activity of the prostaglandin-like material found at 19 h in the challenged joint qualitatively resembled the E-type rather than the F-type since it was far more active on the rat stomach strip and chick rectum than on the rat colon, which is particularly sensitive to $F_{2\alpha}$ (Figure 3). The results of the repeat experiment in which the animals were killed 18 h after challenge, and t.l.c. used to separate the type of prostaglandin present, are shown in Figure 4. Approximately eight times as much E_1 (50 ng) as E_2 (6 ng) was present. Since

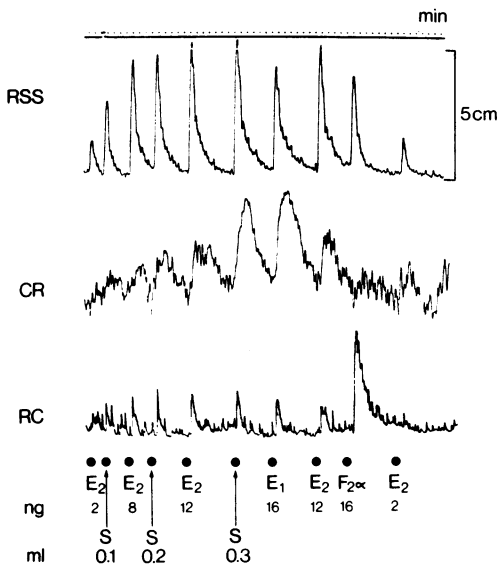

Fig. 3 Identification of prostaglandins in the challenged joints of vehicle-treated Old English rabbits killed 19 h after challenge. Prostaglandins were extracted from the bulked synovial fluid from 5 joints before assay. The test samples (S) or calibrating doses of prostaglandin E_1, E_2 or $F_{2\alpha}$ were superfused over a rat stomach strip (RSS), a chick rectum (CR) and a strip of rat colon (RC). All tissues were treated with mepyramine, methysergide and atropine.

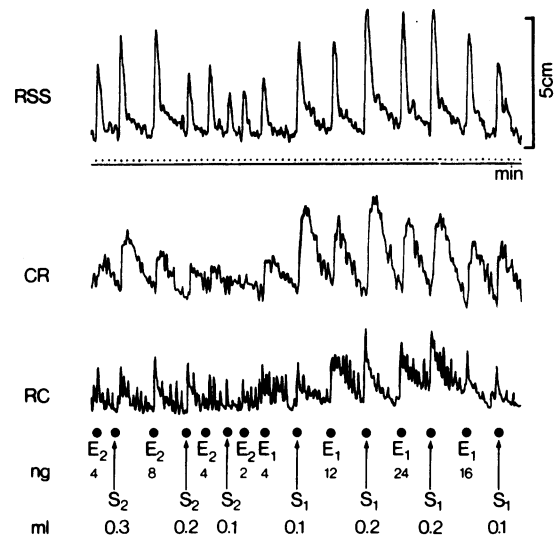

Fig. 4 Bioassay of prostaglandins from the challenged joints of Old English rabbits killed 18 h after challenge. E-type prostaglandins were extracted from the bulked synovial fluid from 10 joints and separated by t.l.c. The test samples containing prostaglandin E_1 (S_1) or prostaglandin E_2 (S_2), or calibrating doses of prostaglandin E_1, E_2 or $F_{2\alpha}$, were superfused over a rat stomach strip (RSS), a chick rectum (CR) and a strip of rat colon (RC). All tissues were treated with mepyramine, methysergide and atropine.

the stomach strip is half as sensitive to E_1 as it is to E_2, this would largely account for the discrepancy with the previous experiment where the mean prostaglandin activity found at 19 h was 19.2 ng assayed as E_2.

Joint and body temperature

Joint temperatures were measured in the group of rabbits killed after 7 days (Figure 5). There was a rise in temperature of between 2-3°C in the challenged knee of the vehicle-treated rabbits which roughly paralleled the course of joint swelling. However, the initial rise was maximal at 24 h and therefore preceded the maximum joint swelling. The temperature of the control knee, although less than that of the challenged knee, also showed an increase in temperature which was maximal at 24 hours. Indomethacin reduced the increase in the temperature of both joints by a similar amount. These results suggested that challenge in one knee joint may produce a systemic hyperthermia, and this was later confirmed in two other groups of rabbits by measuring the rectal temperature 26 h after challenge (Figure 6). Indomethacin also reduced this increase.

Joint histopathology

There was no obvious difference in the histopathology at any time when the rabbits were killed between the challenged joints of the indomethacin-treated and vehicle-treated rabbits. At 6 h the lesion was essentially a vascular reaction characterized by congestion, haemorrhage and thrombosis, and oedema, in particular of the periarticular muscle. The PMN accumulation was limited to the intravascular and the immediate perivascular regions. The 19 h lesion was similar to that of the 6 h lesion, but more severe. The PMNs appeared to be spreading away from the blood vessels and into the surrounding connective tissue and joint space. The joint reaction at 47 h was still essentially a severe, acute inflammation with marked PMN infiltration into the synovium and joint space. A few mononuclear cells were present amongst the infiltrating leucocytes.

At 7 days an acute inflammatory reaction was still present, but areas of chronic inflammation

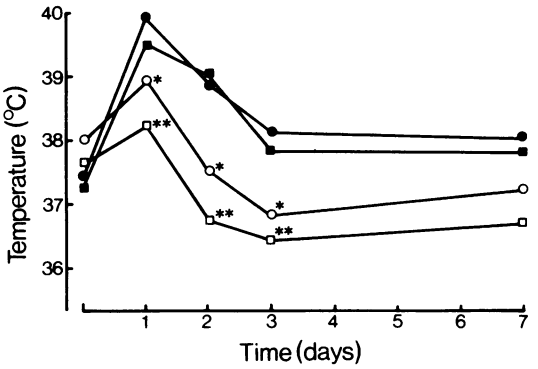

Fig. 5 Effect of indomethacin (7.5 mg/kg) or vehicle twice daily by mouth on surface temperature of challenged and control knee joints of Old English rabbits killed 7 days after challenge. Each point represents the mean of the observations from 4-5 rabbits. (○) challenged joint indomethacin-treated; (●) challenged joint vehicle-treated; (□) control joint indomethacin-treated; (■) control joint vehicle-treated. * $P < 0.05$ for indomethacin versus vehicle on the temperature of the challenged joint. ** $P < 0.05$ for indomethacin versus vehicle on the temperature of the control joint.

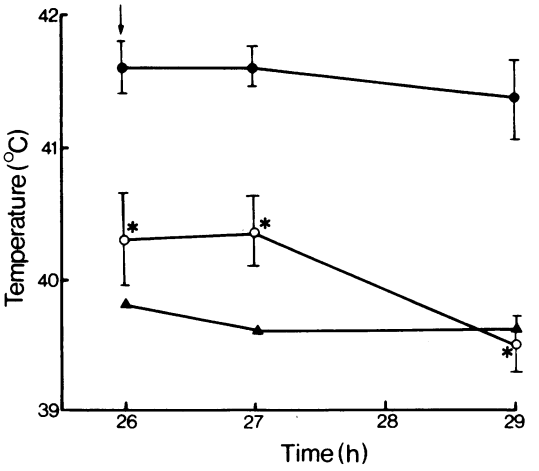

Fig. 6 Effect of indomethacin (7.5 mg/kg) or vehicle by mouth on the rectal temperature of Old English rabbits 26-29 h after challenge. Each point represents the mean of the observations from 8-9 rabbits. (○) indomethacin-treated; (●) vehicle-treated; (▲) normal rabbit (no treatment). Vertical bars are s.e. of mean. * $P < 0.05$ for indomethacin- versus vehicle-treated rabbits. The arrow indicates where indomethacin or vehicle was administered. The rabbits had previously received 2 doses of either indomethacin or vehicle and this probably accounts for the difference in rectal temperature between the two groups at 26 hours.

with fibrosis and mononuclear cells were readily observed. In contrast to the earlier stages, the infiltrating neutrophils had visible granules. On day 46 the synovial reaction was one of chronic inflammation with fibrosis (pannus formation) and plasma cell accumulations as the predominant features. The fibrosis showed variable degrees of extension into the joint space producing erosions of the cartilage and adjacent bone. Neutrophil infiltration was marked in some of the more severely fibrosed joints.

There was a pronounced periarticular reaction at 6 h, 19 h and 47 hours. This could account for the marked joint swelling over this period. At 7 days and 46 days the intra-articular reaction was usually more severe than the periarticular reaction.

Indomethacin plasma levels

Mean plasma concentrations were between 0.5-1 μg/ml indomethacin in all the groups except the group killed at 47 h where it was 3.0 μg/ml (Table 2). These levels are similar to the human therapeutic range (Holt & Hawkins, 1965) and were sufficient to inhibit prostaglandin synthesis in the rabbit as shown by the reduced prostaglandin levels in the joint fluid.

Table 2 Plasma levels of indomethacin in Old English rabbits killed at various times after challenge and oral dosing with indomethacin (7.5 mg/kg) twice daily, the last dose administered 1 h before death

Time of killing	*No. of samples*	*Plasma concentration (μg/ml)*
6 h	5	0.98 ± 0.13
19 h	5	0.77 ± 0.29
47 h	4	3.0 ± 1.24
7 days	4	0.5 ± 0.29
46 days	4	0.5 ± 0.16

Figures are mean ± s.e. mean.

Discussion

Prostaglandins have been found in the synovial fluid of rabbits with monoarticular arthritis. Their presence parallels an increase in joint temperature, but peak prostaglandin levels precede maximum joint swelling and a large increase in both cell infiltration and free acid phosphatase activity. Indomethacin, at the equivalent of human therapeutic concentrations, almost completely inhibited prostaglandin production and this was accompanied by a fall in joint temperature and a moderate reduction in joint swelling. However, it did not significantly reduce cell numbers or enzyme activity, or the histopathological damage seen at the end of the experiment. This suggests that prostaglandins are not of chief importance in the disease process *per se*. Since indomethacin also has an anti-inflammatory effect in rheumatoid arthritis without interfering with the progress of the disease, our results support the suggestion of Vane (1971) that aspirin-like drugs may owe their clinical activity to inhibition of prostaglandin synthetase.

The coincident appearance of peak levels of free acid phosphatase activity, which was used as the lysosomal enzyme marker, and a large rise in numbers of PMNs and maximum joint swelling at 47 h suggests that lysosomal enzymes from PMNs are important mediators of the inflammation. The rise in free acid phosphatase levels is probably the result of increased phagocytosis and this is supported by the fact that visible granules could not be shown in the PMNs before day seven. Much of the pathology seen on day 46 may be due to lysosomal enzyme activity, since similar changes have been produced by repeated injection of lysates of leucocyte lysosomes into rabbit knee joints (Weissmann, Spilberg & Krakauer, 1969). Lysosomal enzymes may also be responsible for the production of joint lesions in rheumatoid arthritis (Dingle, 1962; Weissmann *et al.*, 1969; Muirden, 1972). The failure of indomethacin to affect the gross features of joint damage in rabbit monoarticular arthritis may therefore be due to its inability to prevent the release of lysosomal enzymes. It is also interesting to note that prednisolone has been reported to inhibit joint swelling in this model and to inhibit some of the histopathological signs of inflammation (Davis, 1971). This may be due to the ability of steroids to stabilize lysosomes (Weissmann & Dingle, 1961).

Peak levels of prostaglandins occur at 19 h and may be the result of tissue damage caused by antigen/antibody complexes since marked vascular congestion and haemorrhage, typical of an Arthus reaction, were seen at this time. Prostaglandins have been reported to parallel leucocyte infiltration in many inflammatory conditions including carrageenin air bleb oedema (Anderson, Brocklehurst & Willis, 1971) and rabbit allergic uveitis (Eakins, Whitelocke, Perkins, Bennet & Unger, 1972), but it is not clear whether the prostaglandins are the cause or effect of cell migration. For example Higgs & Youlten (1972) showed that prostaglandins are released from PMNs *in vitro* during phagocytosis. However, we have found that peak levels of prostaglandin occur before a large rise in cell numbers which suggests that prostaglandins are a cause of leucocyte migration. Moreover, E_1, which is chemotactic *in vitro* (Kaley & Weiner, 1971), is the predominant prostaglandin in rabbit monoarticular arthritis. However, indomethacin almost completely blocked prostaglandin production, but had no effect on cell infiltration. It is likely therefore, that other chemotactic agents are produced by the initial antigen/antibody reaction, and these could include complement factors (Ward, 1972), lysosomal enzymes and damaged cells (see review by Sorkin, Stecher & Borel, 1970) and leukoegresin (Yoshida, Yoshinaga & Hayashi, 1968).

Although the presence of large numbers of PMNs in the early stages of rabbit monoarticular arthritis can be explained, the cause of their presence in the chronic stages of the disease is unknown. It may be due to the persistence of antigen in the joint and a low grade immunological response. Cooke & Jasin (1972) have reported, for example, the local production of immunoglobulin to sequestered ovalbumin in the joints of this model. Small amounts of ovalbumin have also been shown to persist for long periods in the synovium (Webb, Ford & Glynn, 1971), synovial macrophages (Webb, Goldberg, Bluestone & Pearson, 1972) and articular cartilage (Cooke, Hurd, Ziff & Jasin, 1972).

Maximum prostaglandin production occurred almost at the same time as maximum joint temperature. Indomethacin reduced both of these as well as the fever in challenged animals. Since prostaglandins are thermogenic in man (Filshie, 1971) and E_1 is a very potent thermogenic agent when injected into the third ventricle of cats (Milton & Wendlandt, 1970) or the cerebral ventricles of cats, rabbits and rats (Feldberg & Saxena, 1971), our results could suggest that E_1 leaks out of the challenged joint and acts centrally to raise the body temperature, including the non-challenged joint. However, this seems unlikely since prostaglandins are rapidly removed from the circulation in rabbits (Ferreira & Vane, 1967).

Another possibility is that leucocyte pyrogen escapes from the joint. Since pyrogen may act via brain prostaglandins (Feldberg, Gupta, Milton &

Wendlandt, 1972) and indomethacin is known to inhibit rabbit brain prostaglandin synthetase (Flower & Vane, 1972), this could explain the effect of indomethacin.

The higher temperature of the challenged joint compared with the control joint is partly due to vasodilatation. Since prostaglandins are known to cause vasodilatation (Crunkhorn & Willis, 1971; Sondergaard & Greaves, 1971; Ferreira, 1972) it is surprising that indomethacin did not cause a greater reduction in the temperature of the challenged knee compared with the non-challenged knee. However, this may be due to the presence of other vasodilators such as histamine or bradykinin which occur in inflammation.

In conclusion, our results suggest that lysosomal enzymes may be more important than prostaglandins in mediating joint swelling and damage to joints. The lack of effect of indomethacin on joint histopathology may be due to its inability to prevent the release of lysosomal enzymes. However, prostaglandins do appear to be causally related to increased joint temperature and to some extent joint swelling. Therefore, our results may go some way to explaining the clinical anti-inflammatory activity of indomethacin and agree with the prediction of Willis, Davison, Ramwell, Brocklehurst & Smith (1972) that prostaglandin E may be involved in the acute inflammatory stage of rheumatoid arthritis.

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note added in proof:

[1] Present address: Department of Pharmacology, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN.